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Attorney for Applicant — Date: May 6, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application Of	:	Group Art Unit 1819
LOUIS D. FALO, JR. ET AL.	:	Examiner J. Schmuck
Serial No. 08/931,219	:	Attorney Docket No. 125350-3
Filed September 19, 1997	:	
Entitled	:	
STIMULATION OF CELL-MEDIATED IMMUNE RESPONSES BY TARGETED PARTICULATE GENETIC IMMUNIZATION	:	

DECLARATION OF LOUIS D. FALO, JR.

I, LOUIS D. FALO, JR., being duly sworn hereby declare as follows:

1. I am a co-inventor of the invention set forth in the claims of the above-captioned application.

2. I received a B.S. Degree in Biochemistry/Chemistry from the University of Pittsburgh (1981); and an M.D./Ph.D. in Immunology from Harvard Medical School and Harvard University (1988).

3. I have held the following post graduate positions: Research Associate (Immunology), Department of Pathology, Harvard Medical School (1987-1988); Resident-PGY1, Department of Medicine, Massachusetts General (1988-1989); Resident, Department of Dermatology, Harvard Medical School (1989-1992); and Research Fellow, Division-Lymphocyte Biology, Dana-Farber Cancer Institute (1990-1992).

4. I am currently the Assistant Professor and Vice Chairman in the Department of Dermatology, University of Pittsburgh School of Medicine, a position that I have held since October 1996. Prior to that time, I was the Assistant Professor, Director of Research and Academic Affairs in the Department of Dermatology, University of Pittsburgh School of Medicine (October 1993 to October 1996), and an instructor in the Department of Dermatology, Harvard Medical School (1992-1993).

5. I have received the following honors and awards: Summa cum laude, B.S., University of Pittsburgh (1981); Senior of the Year, University of Pittsburgh (1981); Phi Beta Kappa (1981); Wellcome Cancer Research Award (1992); and Leadership Award - American Dermatology Association (1996).

6. I have authored or co-authored the publications listed in Exhibit I.

7. I am a member of the following professional societies: American Association for the Advancement of Science (1993 to Present); Society of Investigative Dermatology (1992 to Present); American Association of Immunologists (1995 to Present); and the American Association of Cancer Research (1995 to Present).

8. I participated in the preparation of the above-captioned application and claims, read the same thoroughly before the case was filed, and I have recently re-read and reviewed the application and claims of the case.

9. I have carefully read the Final Rejection dated March 18, 1997 which included an objection to the specification under 35 U.S.C. § 112, first paragraph, for failing to provide an enabling disclosure and a rejection of Claims 1-67 on the same basis. I have also carefully read the Preliminary Amendment to which this is attached.

10. The Final Rejection included an argument that no evidence was presented as to the representative nature of the OVA antigen. The use of OVA antigen is widespread throughout the immunology art, and is accepted by those skilled in the art as being representative of all antigens. An antigen, by definition, is something that is recognized by and stimulates T cells. Because all antigens function in this manner, proof of one antigen would be accepted by those skilled in the art as proof of all antigens.

11. The Final Rejection also included an argument that the mouse tumor model was not representative of all tumors. Numerous researchers in the area of immunology utilize the identical or similar mouse tumor models presented in the evidence submitted with this patent application. Other mouse tumor models are also well used and recognized in the art. The Preliminary Amendment identifies numerous, refereed articles that utilized both the same and different mouse tumor models, as well as use of the OVA antigen. It is my well-considered opinion, based upon my years in the field, that those skilled in the art would accept the evidence provided, utilizing the OVA antigen and the particular mouse tumor model, as providing reliable evidence that the methods of the present invention function in the claimed manner and produce the claimed result.

12. In addition to the experimental data provided in the application and my earlier Declaration, under my direction and control, the following experiment was performed utilizing human skin. This experiment utilized the technology taught in the patent application.

13. Fresh sterilely excised adult human skin was washed, shaved, and separated from subcutaneous tissue. Using the gene gun, two overlapping pulses of 600 PSI were used to deliver the marker gene pCMV:BGal (approximately 2.00 ug of DNA) or the plasmid backbone alone to the human skin. Skin sections were then immediately cultured as skin organ cultures in KGM media. Twenty-four hours later, skin was harvested, fixed in paraformaldehyde, and stained for *B*-galactosidase activity as described by Hengge et al., "Expression of Naked DNA in Human, Pig and Mouse Skin", *J. Clin. Inv.*, Vol. 97, pp. 2911-2916 (1996). As shown in Exhibit II, particles were present throughout the superficial and deep layers of the epidermis. Specific areas on blue staining, indicated *B*-galactosidase expression, were evident within cells in the uppermost layer of the epidermis, as well as in the basal layer. Clearly, not all particles were associated with *B*-galactosidase expressing cells, but in most cases *B*-galactosidase expression were associated with the presence of particles. No staining was found in skin sections shot with plasmid backbone alone (data not shown). Furthermore, areas of expression were noted in the superficial dermis. Thus, the methods of the present invention can be used to elicit DNA expression in human cells.

14. By following the procedures detailed in the patent application and as defined by the pending claims, I would conclude that the disclosed invention teaches that genetic immunization induces potent antigen-specific CTL responses and antigen-specific, CTL-dependent protective tumor immunity and that transfected dendritic cells from the skin will migrate to the lymph nodes, where DNA encoding for a protein is expressed. It is my well-considered opinion that anyone skilled in the art could repeat the methods disclosed in the pending application without undue experimentation to achieve these results. Furthermore, it is my well-considered opinion that one skilled in the art would accept the data presented as representative of being successful in a human, and could practice these methods on a human without undue experimentation, particularly in light of the fact that others have successfully repeated these procedures using different antigens and cells. Therefore, after reviewing this Office Action and the specification, and in light of the results discussed above, it is my further well-considered opinion that the specification is clearly enabling to one skilled in the art and that the pending claims as amended by the attached Preliminary Amendment could be practiced without undue experimentation by such an individual.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

5/5/98
Date

Louis D. Falo, Jr.

EXHIBIT I

PUBLICATIONS

REFEREED ARTICLES:

1. **Falo LD Jr.**, Sullivan K, Benacerraf B, Mescher MF, and Rock KL. Analysis of antigen-presentation by metabolically inactive accessory cells and their isolated membranes. ***Proc. Natl. Acad. Sci. USA***. 1986, 82: 6647-6651.

2. **Falo LD Jr.**, Benacerraf B, and Rock KL. Phospholipase pretreatment of antigen pulsed accessory cells selectively inhibits antigen-specific MHC restricted, but not allospecific stimulation of T lymphocytes. ***Proc. Natl. Acad. Sci. USA***. 1986, 83: 6694-6997.

3. **Falo LD Jr.**, Haber SI, Herrmann S, Benacerraf B, and Rock KL. Characterization of antigen association with accessory cells. I. Specific removal of processed antigens from the cell surface by phospholipases. ***Proc. Natl. Acad. Sci. USA***. 1987, 84: 522-526.

4. **Falo LD Jr.**, Benacerraf B, Rothstein L, and Rock KL. Cerulenin is a potent inhibitor of antigen processing by antigen-presenting cells. ***J. Immunol.*** 1987, 139: 3918-3923.

5. Williams WW, **Falo LD Jr.**, Lu CY, Bennacerraf B, and Sy MS. Effects of in vitro monoclonal anti-I-A antibody treatment in neonatal mice of intrathymic and peripheral Class II antigen expression. ***Cell. Immunol.*** 1988, 111: 126-138.

6. **Falo LD Jr.**, Colarusso, LJ, Benacerraf B, and Rock KL. Serum proteases alter the antigenicity of peptides presented by class I major histocompatibility complex molecules. ***Proc. Natl. Acad. Sci. USA***. 1992, 89: 8347-8350.

7. Razi-Wolf Z, **Falo LD Jr.**, and Reiser, H. Expression and function of the costimulatory molecule B7 on murine langerhans cells. ***Eur. J. Immun***. 1994, 24:805-811

8. **Falo LD Jr.**, Sober AJ, and Barnhill, RL. Evolution of Nevus Spilus. ***Dermatology***. 1994, 189:382

9. **Falo LD Jr.**, Kovacsovics-Bankowski, M, Thompson, K and Rock KL. Targeting antigen into the phagocytic pathway *in vivo* induces protective tumor immunity. ***Nature Medicine***. 1995, 1:649-653.

10. Kress, DW, Seraly, MP, **Falo, LD, Jr.**, Kim B., Jegasothy, BJ, and Cohen B. Olmstead Syndrome. ***Arch Dermatol***. 1996, 132:797-800.

11. Gonzalez S., Vibhagool C, **Falo, LD,Jr.**, Momtaz KT, Grevlink J, and Gonzalez E. Treatment of pyogenic granuloma with the 585 NM pulsed dye laser. ***J. Am. Acad. Dermatology***. 1996, 35:428-431.

12. Mayordomo, JI, Zorina T, Storkus, WJ, Celluzzi, CM, **Falo LD Jr.**, Ildstad ST, Kast MW, DeLeo AB, and Lotze MT. Bone marrow-derived dendritic cells pulsed with tumor peptides effectively treat established murine tumors. ***Nature Medicine.*** 1996, 1:1297-1302.

13. Celluzzi, C, Mayordomo, JI, Storkus WJ, Lotze MT, and **Falo, LD, Jr.**, Peptide-pulsed dendritic cells induce antigen specific CTL-mediated protective tumor immunity. ***J. Exp. Med.*** 1996, 183:283-287.

14. Condon, C, Watkins, S, Celluzzi C., Thompson, K.M, and **Falo, LD, Jr.** DNA-based immunization by *in vivo* transfection of dendritic cells. ***Nature Medicine*** 1996, 2(10):1122-1128.

15. C. Celluzzi and **Falo, LD, Jr.** Epidermal dendritic cells induced potent antigen-specific CTL-mediated immunity. ***J. Invest. Dermatology.*** 108: 716-720. 1997.

16. Celluzzi, C. and **Falo, LD, Jr.** Cutting Edge: Physical interaction between dendritic cells and tumor cells results in an immunogen that induces protective and therapeutic tumor rejection. ***J. Immunol.(Cutting Edge).*** 160: 3081-3085. 1998.

17. Deng, J-S, **Falo, LD, Jr.**, Kim, B., and Abell, E. Cytotoxic T-cells in Basal Cell Carcinomas of the Skin. ***American Journal of Dermatolopathology. In Press.*** 1998.

18. Ling MR, et al., A placebo-controlled, randomized, double-blind trial of once-weekly fluconazole for 4, 6, or 9 months of treatment for distal subungual onychomycosis of the toenail. ***J. Am. Acad. Dermatology. In Press.*** 1998.

19. Tuting, T., Storkus, W.J., and **Falo, LD, Jr.**. DNA Immunization targeting the skin: molecular control of adaptive immunity. *(submitted). 1998.*

20. L. Geskin, C. Celluzzi, and **L.D. Falo, Jr.** In vivo trafficking of adoptively transferred dendritic cells. (***in preparation***). 1998.

21. C. Condon, K. Thompson, and **L.D. Falo, Jr.** Internalization, processing, and MHC Class I restricted presentation of particulate antigens by keratinocytes. (***in preparation***). 1998.

22. **Falo, L.D., Jr.**, Plowey, J., and Thompson, K. Processing and presentation of particulate antigens by dendritic cells. (*in preparation*). 1998.

23. B. Amos, **L.D. Falo, Jr.**, E. Abell, and J-S Deng. Evidence for activated cytotxic T-lymphocytes in regressing halo nevi. (*in preparation*). 1998.

REVIEWS, INVITED PUBLISHED PAPERS, PROCEEDINGS OF CONFERENCES AND SYMPOSIA, MONOGRAPHS, BOOKS, AND BOOK CHAPTERS:

1. Rock KL, **Falo LD Jr.**, and Benacerraf B. Antigen-presentation of Ir gene controlled amino acid copolymers. In: ***Immunogenecity of Protein Antigens: Repertoire and Regulation.*** (Sercarz E and Berzofsky J, eds.), CRC PRES. 1986.

2. Benacerraf B, **Falo LD Jr.**, and Rock KL. Processing of native antigen by accessory cells and presentation of membrane bound MHC associated antigen to specific T cells. In: ***"Processing and Presentation of Antigen"***. (Vogel, Pernis, and Silverstein Eds,) Biomedical Sciences Symposium, June 1986.

3. Rock KL, **Falo LD Jr.**, Benacerraf B, and Abbas A. Processing and presentation of antigens to MHC restricted T lymphocytes. ***Annales de l'Institut Pasteur***. 1986.

4. Rock KL, **Falo LD Jr.**, Michalek M, Rothstein L, and Benacerraf B. Analysis of the association of processed antigen with the APC surface. In: ***The T-Cell Receptor***. M.M. Davis and J. Kappler,eds.) ARL, Inc., New York. *1987.*

5. Haynes HA, **Falo LD Jr.**, and Hood AF. Dermatologic Complications of Cancer Chemotherapy Including Alopecia. In: ***Cancer Medicine***, 3rd Edition. (Editors, Holland F. Erie E, Bast RC, Kufe DW, Morton DL, Weichselbaum RR) Lea & Febiger, Malvern PA. *1993.*

6. **Falo LD Jr.**, and Lotze MT. Cancer Vaccines. In ***Manual of Clinical Laboratory Immunology***, *5th Edition.* Chapter 133. Volume Editor: E.C. de Macario. Saunders. *1997.*

7. Tuting, T., Austin, J., Storkus, W. And **Falo LD Jr.** The Immunology of DNA Vaccines. *In DNA Vaccines: Methods and Protocols*, for the Series ***Methods in Molecular Medicine***, (Editors, Lowrie, D., and Whalen, R.) Humana Press, Inc., *In press, 1998.*

8. Barrett-Boyes, S.,and **Falo LD Jr.**. Dendritic Cell Trafficiking. In ***Dendritic cells: Biology and Clinical Application*** (Editors, Lotze, M., and Thompson, A.) Academic Press. *In press. 1998.*

9. **Falo LD Jr.** Genetic Immunization By *in vivo* Transduction of Dendritic Cells. In ***Gene Therapy of Cancer: Methods and Protocols***, (Editors, Walther, W and Stein, U.)Humana Press, Inc. *Invited, In preparation. 1998.*

10. **Falo LD Jr.** Cutaneous Genetic Engineering of the Immune Response. ***Proceedings of the Association of American Physicians***. *Invited, in Preparation, 1998.*

11. **Falo LD Jr.** *Editor for* "Antigen Delivery Strategies for Immunization", ***Advanced Drug Delivery Reviews***. Elsevier Publications. *Invited, in preparation, 1999.*

BOOK REVIEW:

1. **Falo LD Jr.** Concepts in Vaccine Design (edited by S. H. E. Kaufman). Walter de Gruyter & Co. in ***Nature Medicine*** 3(1): 95. 1997.

EXHIBIT II